

FROMMER LAWRENCE & HAUG LLP

745 Fifth Avenue
New York, New York 10151
Telephone: (212) 588-0800
Facsimile: (212) 588-0500
E-mail: Firm@flhlaw.com

FACSIMILE COVER LETTER

To: Examiner: Shin Lin Chen Group: 1632
Firm: U.S.P.T.O.
Facsimile: 571-273-0726
From: Marilyn Matthes Brogan
Date: February 24, 2005
Re: Applicant: Loerz et al
Application No.: 09/674,824
Filed: November 6, 2000
For: NUCLEIC ACID MOLECULES WHICH CODE FOR ENZYMES
DERIVED FROM WHEAT AND WHICH ARE INVOLVED IN THE
SYNTHESIS OF STARCH
Our Ref. No. 514413-3848

Number of Pages: 2
(including cover page)

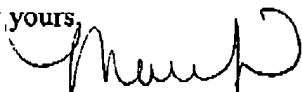
If you do not receive all pages or are unable to read the transmission, please call and ask for Anna (Ext. 2068).

Dear Mr. Chen:

Further to our earlier conversation of today, attached is a copy of page 30 of the specifications to the above referenced matter.

Best regards.

Very truly yours,



Marilyn Matthes Brogan

CONFIDENTIALITY NOTICE

The documents accompanying this transmission contain confidential information intended for a specific individual and purpose. The information is private, and is legally protected by law. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution, or the taking of any action in reliance on the contents of this facsimile is strictly prohibited.

00258454

WO 99/58688

30

PCT/EP99/03156

The method for transforming immature wheat embryos was developed and optimized by Becker and Lörz (D. Becker and H. Lörz, Plant Tissue Culture Manual (1996), B12: 1 to 20).

- 5 In the experiments described hereinbelow, the procedure developed by Becker and Lörz (loc. cit.) was adhered to.

10 For the transformation, ears with caryopses of developmental stage 12 to 14 days after anthesis were harvested and surface-sterilized. The isolated scutella were plated onto induction medium #30 with the embryo axis orientated towards the medium.

15 After preculture for 2 to 4 days (26°C, in darkness), the explants are transferred to medium #39 for the osmotic preculture (2 to 4 h, 26°C, in the dark).

20 For the biolistic transformation, approx. 29 µg of gold particles onto which a few µg of the target DNA had previously been precipitated were employed per shot. Since the experiments carried out are cotransformations, the target DNA added to the precipitation batch is composed of the target gene and a resistance marker gene (bar gene) in the ratio 1:1.

4. DIG labeling of DNA fragments

- 25 DNA fragments employed as screening probes were labeled via a specific PCR with the incorporation of DIG-labeled dUTP (Boehringer Mannheim, Germany).

Media solutions used in the examples:

30

20 × SSC 175.3 g NaCl
 88.2 g sodium citrate
 twice-distilled H₂O to 1000 ml
 10 N NaOH to pH 7.0

35

Plasmid pTaSSI 8/1 was deposited at the DSMZ in Braunschweig, Federal Republic of Germany, as specified in the Budapest Treaty under the No. DSM 12794.

The method for transforming immature wheat embryos was developed and optimized by Becker and Lörz (D. Becker and H. Lörz, Plant Tissue Culture Manual (1996), B12: 1 to 20).

- 5 In the experiments described hereinbelow, the procedure developed by
 . Becker and Lörz (loc. cit.) was adhered to.

For the transformation, ears with caryopses of developmental stage 12 to 14 days after anthesis were harvested and surface-sterilized. The isolated scutella were plated onto induction medium #30 with the embryo axis orientated towards the medium.

After preculture for 2 to 4 days (26°C, in darkness), the explants are transferred to medium #39 for the osmotic preculture (2 to 4 h, 26°C, in the dark).

For the biolistic transformation, approx. 29 µg of gold particles onto which a few µg of the target DNA had previously been precipitated were employed per shot. Since the experiments carried out are cotransformations, the target DNA added to the precipitation batch is composed of the target gene and a resistance marker gene (bar gene) in the ratio 1:1.

4. DIG labeling of DNA fragments

- 25 DNA fragments employed as screening probes were labeled via a specific PCR with the incorporation of DIG-labeled dUTP (Boehringer Mannheim, Germany).

Media solutions used in the examples:

30

20 × SSC

175.3 g NaCl

88.2 g sodium citrate

twice-distilled H₂O to 1000 ml

10 N NaOH to pH 7.0

35

Plasmid pTaSSI 8/1 was deposited at the DSMZ in Braunschweig, Federal Republic of Germany, as specified in the Budapest Treaty under the

see the ~~newly~~ boxed
page 30 for the missing
portion
35
missing
part.
Plasmid pTaSSI
Republic of Gen